Peruvian maize resistance to European corn borer - A preliminary report

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Research Objectives

1) Introgress Peruvian maize European corn borer resistance into B94 and B97.

2) Determine the mechanism of resistance for the Peruvian maize resistance factor (PMRF)

3) Determine if improved breeding populations contain resistance to multiple maize pests.

Introduction

The European corn borer, Ostrinia nubilalis (Hübner) (ECB), is a serious economic pest in the United States (Barry, 1989). A bivoltine strain of ECB, predominant throughout the northern Corn Belt, damages the maize plant at two distinct stages. First generation ECB (ECB1) damage is characterized by leaf-feeding in the whorl of the plant. Second generation ECB (ECB2) damage is characterized by leaf-sheath feeding, collar feeding, stalk tunneling, and ear shank feeding (Showers et al., 1989).

ECB larvae feed cryptically within the whorl and stalk of the maize plant which reduces the effectiveness of chemical control and increases the importance of developing maize resistant to ECB larval feeding (Barry and Darrah, 1991).

ECB leaf-feeding resistance in maize has been identified and primarily attributed to the chemical (DIMBOA) (Klun et al., 1967). Breeding programs for this trait have been successful, however, highly resistant DIMBOA-based hybrids did not yield as well as more susceptible hybrids. Cultivar development efforts focused on improvement of elite lines for yield and standability at the expense of developing ECB resistance. Prior to the use of transgenic maize resistant to ECB, most maize hybrids contained some DIMBOA-based resistance to ECB, however, damage by the insect was still of economic significance during most years (McLeod, 1992; Rice et al., 1996).

The demand for maize hybrids containing ECB resistance has driven the development of transgenic maize expressing crystal protein (*cry*) genes from *Bacillus thuringiensis*. Transgenic maize has shown promise for the control of ECB. A potential problem is the evolution of resistance in target pests (e.g. ECB) (Tabashnik, 1997). Current target pest resistance management concepts show promise but are theoretical and not based on empirical data.

Research identifying unique sources of conventional host plant resistance to ECB in maize has not discontinued. Eleven accessions of Peruvian maize were identified as resistant to ECB1 that was not mediated by DIMBOA (Abel et al., 1995) (Table 2). Wilson et al. (1995) evaluated the 11 resistant Peruvian maize accessions for multiple pest resistance and identified 4 accessions containing resistance to corn earworm, western corn rootworm, and sugarcane borer, and 7 accessions highly resistant to ECB2.

The objectives of this research program are: 1) introgress the PMRF trait into B94 and B97, 2) determine the mechanism of resistance of PMRF, and 3) determine if improved breeding populations contain resistance to multiple maize pests.

Research Procedures and Results

I. Introgression of PMRF trait into B94 and B97

Selection of the parents

A backcrossing program was conducted to introgress the Peruvian maize ECB resistance trait into two elite U. S. Corn Belt inbreds. The donor parents were all Plant Introductions (Table 2) selected for their high levels of resistance to ECB1 (Abel et al., 1995) and ECB2 (Wilson et al., 1995).

The recurrent parents were selected after discussing the performance of public inbreds in single cross experiments with Arnel Hallauer (Distinguished Professor, Agronomy Dept., Iowa State University, Ames, Iowa), Linda Pollak (Research Geneticist, USDA-ARS Corn Insects and Crop Genetics Research Unit), and Wilfredo Salhuana (Retired Research Fellow, Pioneer Hi-Bred International, Inc., Miami, Florida). B94 (Russell, 1991) and B97 (Hallauer et al., 1994) were chosen for their high yield performance in single cross experiments. B94 belongs to the Stiff Stalk heterotic group. B97 belongs to the non-Stiff Stalk heterotic group. The Peruvian maize was crossed to both B94 and B97 because the heterotic group of the Peruvian maize accessions were unknown.

Backcross breeding program

Table 1 discusses the procedures used during the backcross breeding program.

Table 1. Breeding program used to introgress PMRF into B94 and B97.

Season and Location	**Crosses and Procedures**
Winter, Salinas, Puerto Rico, 1994	Peruvian maize x B94 or B97 Eleven ECB resistant Peruvian maize Plant Introductions (Table 2) were crossed, with reciprocals, to B94 or B97. The first crosses were made at Salinas because the Peruvian maize accessions are photoperiod sensitive, short day flowering populations. Multiple crosses were made because of the genetic variability within each Peruvian maize accession. Two rows of either B94 or B97 were grown next to one row of a Peruvian maize accession. One row of an inbred was planted at the same time as the Peruvian maize accession and another row of the same inbred was planted 4 days later. Each 12' row contained ~16 plants. Plant to plant crosses were made when the inbred was used as the male parent. When the Peruvian maize accessions were used as male parents to cross with the inbred, the pollen was bulked from several plants within a row to have better representation of the landrace. Rows were harvested separately in balanced bulk. One hundred thirteen Peru x B94 rows were harvested and 100 Peru x B97 rows were harvested. Packets of seed containing approximately 100 seeds from each row were shipped to Ames.

Season and Location	Crosses and Procedures
Summer, USDA/ARS North Central Regional Plant Introduction Station (NCRPIS), Ames, IA, 1994	F1 x B94 or B97 Thirty-five seeds from each F1 were planted (May 10) in 20' x 3' rows. Ten foot alleyways were used between ranges. The (Peru x B94) F1 rows and the (Peru x B97) F1 rows were planted in separate blocks. Rows were thinned to 25 plants per row. Forty-eight, 20' rows of B94 or B97 were grown at the end of each block to be used as the male parents. B94 and B97 were planted at three different planting dates (5/10, 5/20, and 5/31) to nick with the flowering time of the F1 plants. Whorl stage (V 5-6) plants were infested with 250 ECB larvae using an inoculator developed by Mihm (1983). Four weeks after infesting, plants were evaluated for leaf feeding damage using a 9-class rating scale developed by Guthrie et al. (1960). Susceptible and intermediate in resistant plants were discarded. The ECB1 resistant plants that remained were crossed with B94 or B97 to produce BC1:F1 seed. At anthesis, each selected plant was infested with 250 ECB larvae for ECB2 damage testing. Before harvest, plant stalks and ears were tagged individually to trace harvested ears back to the stalk they came from. After harvest, plant stalks were cut in half using a portable ban saw and evaluated for ECB2 stalk tunneling. Seed from resistant plants were saved. Seed from susceptible and intermediate in resistant plants were discarded. Throughout the growing season, whenever rows were weeded, infested with ECB, rated for damage, or cross pollinated, diseased plants and off-type plants were removed from the rows. F1 plants that flowered more than 21 days later than their inbred parent (B94 or B97) were discarded. Plants with a 1 ECB1 rating and /or <2.5" of ECB2 tunneling were harvested individually. Other resistant plants were harvested in bulk within rows with an equal number of seeds harvested per ear.

Season and Location	Crosses and Procedures
Summer, NCRPIS, Ames, IA, 1995	BC1:F1 x B94 or B97 The same selection and backcross procedure used to produce BC1:F1 seed was used to produce BC2:F1 seed. BC1:F1 plants that flowered more than 14 days later than their recurrent parent (B94 or B97) were discarded. Plants were harvested separately.
Summer, NCRPIS, Ames, IA, 1996	BC2:F1 self Twenty five seeds from each BC2:F1 population were planted in fifteen foot rows. Rows were thinned to 18 plants per row. Off type and diseased plants were removed. The earliest maturing plants were selfed. Selfed ears were harvested individually. One hundred seed from 100 BC2:S0 ears containing B94 as the recurrent parent and 100 BC2:S0 ears containing B97 as the recurrent parent were sent to winter nursery to be used in testcrosses.
Winter, Ponce, Puerto Rico, 1997	BC2S0 x Tester A or Tester B BC2:S0 populations were grown in rows in an isolation plot. BC2:S0 with B94 as the recurrent parent were crossed with a private non-Stiff Stalk tester (Tester A). BC2:S0 with B97 as the recurrent parent were crossed with a private Stiff Stalk tester (Tester B).
Summer, 1997, 8 U.S. Corn Belt Locations	Testcrosses (Winter, 1997) were evaluated for yield at 8 locations. Cooperator names and locations for the yield trial are given in Table 3.
Summer, NCRPIS, Ames, IA, 1997	BC2:F1 x B94 or B97 The same selection and backcross procedure used to produce BC1:F1 and BC2:F1 seed was used to produce BC3:F1 seed. Individual plants were infested with 600 ECB to increase the selection pressure for this last generation of backcrossing. BC2:F1 plants that flowered 7 days after flowering of the respective recurrent parent were discarded. Summarized results from ECB2 evaluations of BC2:F1 populations are given in Table 4.

II. PMRF mechanism of resistance

Studies have been conducted to determine the PMRF mechanism of resistance. It appears that PMRF is a deterrent (antixenosis) to ECB larval feeding. Another mechanism of PMRF resistance is antibiosis. PMRF lengthens larval development times and increases larval mortality.

Robbins et al. (1997) found ECB resistance activity in water-based extracts taken from freeze-dried whorl tissue from the Peruvian maize. A study on the chemical(s) responsible for PMRF is being conducted by Craig A. Abel, Richard L. Wilson, and Mark A. Berhow (Research Chemist, USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL).

III. Multiple pest resistance in selected breeding populations

A third objective was to evaluate selected BC2:F1 populations for multiple pest resistance. Sixteen BC2:F1 populations that showed superior ECB resistance were selected for this study. These 16 BC2:F1 populations were sent to the following cooperators to test for multiple pest resistance: fall armyworm (FAW) and corn earworm (CEW), Bill Wiseman, Tifton, GA; western corn rootworm (WCRW), John Foster, Lincoln, NE; sugarcane borer (SCB), Bill White, Houma, LA; ECB oviposition (ECBo), Brad Binder, Ames, IA; ECB1 and ECB2, Richard Wilson and Craig Abel, Ames, IA.

Those maize populations that were resistant to three pests are given in Table 5. ECB2 results have not been analyzed and data from the western corn rootworm test are forthcoming. So far, 4 of the 16 BC2:F1 populations tested contain resistance to three or more maize pests. These maize populations with multiple pest resistance could be useful for developing Integrated Pest Management systems for maize.

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Table 2. European corn borer leaf-feeding ratings and mean levels of MBOA for 11 Peruvian and 2 check maizes (Abel et al., 1995).

Accession identifier	Mean leaf-feeding ratings[abc]	mg MBOA/ g dried whorl tissue	Peruvian maize race (Grobman et al., 1961) or resistance of inbred check
WF9	7.1a	0.217b	Susceptible check
P.I. 503725	3.2 bc	0.210b	Mochero
P.I. 503806	3.2 bc	0.175b	Alazan
P.I. 503849	3.2 bc	0.208b	Alazan
P.I. 503764	3.0 bcd	0.144b	Mochero
Ames-10623	3.0 bcd	0.232b	Arizona
P.I. 503720	2.9 bcd	0.377b	Mochero
P.I. 503722	2.9 bcd	0.274b	Mochero
P.I. 503728	2.9 bcd	0.165b	Mochero
P.I. 503727	2.8 cd	0.276b	Mochero
P.I. 503723	2.6 cde	0.214b	Mochero
P.I. 503731	2.2 de	0.199b	Mochero
CI31A	1.8 e	1.104a	Resistant check

[a] Means followed by the same letter are not significantly different according to the LSD test (P = 0.05)
[b] Guthrie et al. (1960) 1-9 rating scale. 1-3 = resistance; 4-6 = intermediate in resistance; 7-9 susceptible.
[c] Values represent the mean of 8 replications.

Table 3. Yield trial cooperators by replication.

Experiment #	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8 (Disease)
97540	GEM	Pioneer	Golden Harvest	ICI	DeKalb	NC+	Jung Farms	Cargill
97541	GEM	Pioneer	ICI	Holdens	DeKalb	Wyffels	Jung Farms	ICI
97542	GEM	Pioneer	ICI	DeKalb	NC+	Lima-grain	Jung Farms	Grow-mark
97543	GEM	Pioneer	Cargill	Golden Harvest	ICI	DeKalb	Jung Farms	Golden Harvest
97544	GEM	Pioneer	ICI	DeKalb	NC+	Lima-grain	Jung Farms	NC+
97550	GEM	Pioneer	ICI	Bojac	Wyffels	Lina-grain	Jung Farms	Cargill
97551	GEM	Pioneer	ICI	Hoege-meyer	Wyffels	Lina-grain	Jung Farms	Holdens
97552	GEM	Pioneer	Cargill	ICI	Holdens	Great Lakes	Jung Farms	DeKalb
97553	GEM	Pioneer	ICI	Jung Farms	NC+	Great Lakes	Jung Farms	ICI
97554	GEM	Pioneer	ICI	Bojac	Great Lakes	Jung Farms	Jung Farms	Holdens

Table 4. BC2:F1 ECB2 stalk tunneling damage, Ames, 1997.

(Peru P.I. x B94) // B94		(Peru P.I. x B97) // B97	
Maize Population	Inches of ECB stalk tunneling	Maize Population	Inches of ECB stalk tunneling
Transgenic	0.9"	Transgenic	0.7"
B52	2.3"	B52	1.5"
(Ames-10623 x B94) // B94 - - 109/8/5	5.1"	(PI 503720 x B97) // B97 - - 116/B/2	2.6"
(PI 503731 x B94) // B94 - - 81/9/11	5.7"	(PI 503849 x B97) // B97 - - 199/5/5	2.9"
(Ames-10623 x B94) // B94 - - 112/B/5	5.9"	(PI 503727 x B97) // B97 - - 157/B/3	3.0"
(PI 503849 x B94) // B94 - - 107/8/6	6.2"	(PI 503849 x B97) // B97 - - 199/5/7	3.1"
(Ames-10623 x B94) // B94 - - 109/15/2	7.1"	(PI 503764 x B97) // B97 - - 181/B/9	3.3"
B94	29.1"	B97	10.7"
CI31A	25.2"	CI31A	22.8"
WF9	25.9"	WF9	23.3"

Stalk tunneling values represent the mean of approximately 16 plants for the BC2:F1 populations and approximately 28 plants (~7 plants x ~4 rows) for the checks.

PI 503720 is Lambayeque 29.
PI 503727 is Lambayeque 40.
PI 503731 is Lambayeque 45.
PI 503764 is Lambayeque 103.
PI 503849 is Piura 208.
Ames-10623 is Libertad 3.

Table 5. BC2:F1 populations containing resistance to three or four maize pests.

Population	FAW	CEW	SCB	ECBo	ECB1
(PI 503720 x B97) // B97[1]	R		I		R
(PI 503849 x B94) // B94[2]	R	R	I		R
(PI 503731 x B94) // B94[3]		R	I		R
PI 503849 x B94) // B94[4]		R	I	R	R

R = High resistance I = Intermediate resistance.

FAW = fall armyworm, CEW = corn earworm SCB = sugarcane borer, ECBo = ECB oviposition.

[1] PI 503720 is Lambeyeque 29. Backcross identifier is 116/B/10
[2] PI 503849 is Piura 208. Backcross identifier is 100/R/3
[3] PI 503731 is Lambayeque 45. Backcross identifier is 81/9/2 and 81/9/11 equally represented in bulk.
[4] PI 503849 is Piura 208. Backcross identifier is 107/1/7.

Data for Western corn rootworm and ECB2 resistance have not been analyzed.